



PHYTOCHEMICAL, PHARMACOLOGICAL ACTIVITY  
AND MOLECULAR DOCKING INVESTIGATIONS OF  
*Tetracera indica* (Christm. & Panz.) Merr., *Averrhoa*  
*bilimbi* Linn. AND *Gymnanthemum glaberrimum*  
(Welw. ex O.Hoffm.) H. Rob.

BY

ALHASSAN MUHAMMAD ALHASSAN

A thesis submitted in fulfillment of the requirement for the  
degree of Doctor of Philosophy in Pharmaceutical Sciences  
(Pharmaceutical Chemistry)

Kulliyyah of Pharmacy  
International Islamic University Malaysia

APRIL 2018

## ABSTRACT

*Tetracera indica* and *Averrhoa bilimbi* are used in traditional treatment of diabetes mellitus while *Gymnanthemum glaberrimum* is used as a remedy for skin cancer. The objectives of the present study were to investigate the pharmacological activity of leaves of *T. indica*, *G. glaberrimum* and *A. bilimbi*, and their phytochemical constituents. Ethanol extract from the leaves of *T. indica* was subjected to  $\alpha$ -glucosidase inhibitory activity evaluation and isolation of bioactive compounds followed by molecular docking. Methanol extract of *G. glaberrimum* leaves was subjected to cytotoxic investigations on A375, HT-29 and MCF7 cancer cell lines, followed by isolation of bioactive constituents and molecular docking. Purified compounds were characterized using spectroscopic analysis. Furthermore, the antioxidant activity of methanol extract of *A. bilimbi* leaves and its fractions was investigated and the active compounds were identified by LC-MS-QTOF analysis. Nine compounds were isolated from *T. indica* leaves which include; lupeol, betulinic acid, wogonin, norwogonin, quercetin, tectochrysin, kaempferol and two new sulphated flavones viz. 5,7-dihydroxyflavone-O-8-sulphate and 5-hydroxy-8-methoxyflavone-O-7-sulphate. Quercetin, kaempferol and 5,7-dihydroxyflavone-O-8-sulphate showed significant ( $P < 0.05$ )  $\alpha$ -glucosidase inhibitory activity with  $IC_{50}$  values of  $61.86 \pm 2.4$ ,  $68.46 \pm 3.5$  and  $133.57 \pm 5.2$   $\mu$ M respectively, compared to acarbose ( $IC_{50}$   $419.42 \pm 20.29$   $\mu$ M). Molecular docking result showed favourable mode of interactions of these flavonoids in the active site of  $\alpha$ -glucosidase. Furthermore, four compounds viz. nonacosanoic acid, lupeol, 5-methylcoumarin-4- $\beta$ -glucoside and 4-hydroxy-5-methylcoumarin were isolated from *G. glaberrimum*. Lupeol displayed significant ( $P < 0.05$ ) antiproliferative effect against MCF-7 cell lines with  $IC_{50}$  of  $34.15 \pm 2.32$   $\mu$ g/mL. Lupeol and 5-methylcoumarin-4- $\beta$ -glucoside displayed moderate cytotoxic effect on A375 cells with  $IC_{50}$  of  $59.18 \pm 2.70$  and  $91.84 \pm 6.78$   $\mu$ g/mL. 4-Hydroxy-5-methylcoumarin, and lupeol displayed significant ( $P < 0.01$ ) cytotoxic effect on HT-29 cells with  $IC_{50}$  of  $4.22 \pm 0.13$ ,  $15.24 \pm 1.15$   $\mu$ g/mL respectively, which is comparable with 5-fluorouracil ( $IC_{50}$   $8.00 \pm 0.78$   $\mu$ g/mL). CDK2 receptor and CA IX and XII were identified through molecular docking as potential target for these compounds. The n-butanol fraction of *A. bilimbi* extract displayed significant ( $P < 0.05$ ) DPPH radical scavenging effect with  $IC_{50}$  ( $4.14 \pm 0.21$   $\mu$ g/mL). It also exhibited significant ( $P < 0.05$ ) XO inhibitory effect with  $IC_{50}$  of  $64.84 \pm 3.93$   $\mu$ g/mL. Afzelechin 3-O- $\alpha$ -L-rhamnopyranoside and cucumerin A were identified through LC-MS-QTOF as possible metabolites responsible for the antioxidant activity of the n-butanol fraction.

## خلاصة البحث

نباتات تيتراسيرا انديكا و ايفوريا بلجي يستخدمان شعبيا لعلاج داء السكر أما نبات جمنازيمم جلابيريمم يستخدم كعلاج لسرطان الجلد. أهداف الدراسة الحالية هي دراسة النشاط الدوائي لتلك النباتات ومكوناتها الكيميائية. تم فحص فاعلية مستخلص الإيثانول من أوراق تيتراسيرا انديكا في تثبيط انزيم الالفا غليجوزيز وتم عزل المركبات الفعالة. في حين تم التحقق من سمية مستخلص الميثانول من أوراق جمنازيمم جلابيريمم باستخدام خطوط الخلايا السرطاني A375، HT-29، MCF7، تليها عزل المكونات النشطة بيولوجيا. تم وصف المركبات النقية باستخدام التحليل الطيفي. تم دراسة النشاط المضاد للأوكسدة لمستخلص الميثانول من أوراق ايفوريا بلجي ومشتقاتها وتم تحديد المركبات النشطة بواسطة تحليل LC-MS-QTOF. تم عزل تسعة مركبات من أوراق تيتراسيرا انديكا والتي تشمل لوبيول وحمض البوتيلينيك ،وجونين ، نوروجونين ، كيرسيتين ، تيكوكيرسين ، كيميغول بالإضافة الى اثنين من مركبات الفلافون الكيريتية وهما 5،7-ديهيدروكسي فلافون-ثمانى الكيريت و 5-هيدروكسي ميثوكسي فلافون-سباعي الكيريت. أظهر كيرسيتين و كيميغول و 5،7-ديهيدروكسي فلافون-ثمانى الكيريت نشاط تثبيط الالفا غليجوزيدز ( $P < 0.05$ ) مع قيم  $IC_{50}$  من  $2.4 \pm 61.86$  و  $3.5 \pm 68.46$  و  $133.57 \pm 5.2$   $M\mu$  على التوالي ، مقارنة مع. أكاربوز ( $IC_{50} 419.42 \pm 20.29 M\mu$ ). أظهرت النتائج طريقة مواتية للتفاعلات بين هذه الفلافونيدات في الموقع النشط ل الالفا غليجوزيز وعلاوة على ذلك ، تم عزل أربعة مركبات من نبات جمنازيمم جلابيريمم وهم حمض نوناكسانويك، لوبيول ، 5-ميثيل كومارين 4- بيتا جلوكوزايد و 4- هيدروكسي 5- ميثيل كومارين. أظهر لوبيول تأثير معتبر ( $P < 0.05$ ) ضد خطوط الخلية MCF-7 مع  $IC_{50}$  من  $2.32 \pm 34.15$  ميكروغرام/مل. وكذلك أظهر كل من لوبيول و 5-ميثيل كومارين 4- بيتا جلوكوزايد تأثير متوسط ضد خلايا A375 مع  $IC_{50}$  من  $2.70 \pm 59.18$  و  $91.84 \pm 6.78$  ميكروغرام / مل. بالإضافة إلى ذلك فقد أظهر كل من لوبيول و 4- هيدروكسي 5- ميثيل كومارين تأثير ( $P < 0.05$ ) سام للخلايا على HT-29 مع  $IC_{50}$  من  $0.13 \pm 4.22$  ،  $1.15 \pm 15.24$  ميكروغرام / مل على التوالي ، والتي يمكن مقارنتها مع 5-فلورويوراسيل (ميكروغرام / مل  $IC_{50}$  8.00) .  $0.78 \pm$  كذلك تم تحديد مستقبلات CDK2 و CA IX و XII من خلال الالتحام الجزئي كهدف محتمل لهذه المركبات. أظهر n-بيوتانول من مستخلص ايفوريا بلجي تأثير جذري DPPH ( $P < 0.05$ ) مع  $IC_{50}$  ( $0.21 \pm 4.14$  ميكروغرام / مل). كما أظهر تأثيرا كبيرا ( $P < 0.05$ ) ولكن أقل من  $IC_{50}$  ( $64.84 \pm 3.93 \mu g / mL$ ) XO. أخيرا تم تحديد كيومارين A وافزيليسين 3- الفا رامنو بيرانونزايد من خلال LC-MS-QTOF كنواتج ممكنة مسؤولة عن النشاط المضاد للأوكسدة لمركب n-بيوتانول.

## **APPROVAL PAGE**

The thesis of Alhassan Muhammad Alhassan has been approved by the following:

---

Qamar Uddin Ahmed  
Supervisor

---

Alfi Khatib  
Co-Supervisor

---

Siti Zaiton Mat So'ad  
Internal Examiner

---

Jamia Azdina Jamal  
External Examiner

---

Zainul Amiruddin Zakaria  
External Examiner

---

Siti Aesah @ Naznin Muhammad  
Chairman

## DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Alhassan Muhammad Alhassan

Signature .....

Date .....

**INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA**

**DECLARATION OF COPYRIGHT AND AFFIRMATION OF  
FAIR USE OF UNPUBLISHED RESEARCH**

**PHYTOCHEMICAL, PHARMACOLOGICAL ACTIVITY AND  
MOLECULAR DOCKING INVESTIGATIONS OF *Tetracera  
indica* (Christm. & Panz.) Merr., *Averrhoa bilimbi* Linn. AND  
*Gymnanthemum glaberrimum* (Welw. ex O.Hoffm.) H. Rob.**

I declare that the copyright holders of this thesis are jointly owned by Alhassan  
Muhammad Alhassan and IIUM.

Copyright © 2018 Alhassan Muhammad Alhassan and International Islamic University Malaysia.  
All rights reserved.

No part of this unpublished research may be reproduced, stored in a retrieval  
system, or transmitted, in any form or by any means, electronic, mechanical,  
photocopying, recording or otherwise without prior written permission of the  
copyright holder except as provided below

1. Any material contained in or derived from this unpublished research  
may be used by others in their writing with due acknowledgement.
2. IIUM or its library will have the right to make and transmit copies  
(print or electronic) for institutional and academic purposes.
3. The IIUM library will have the right to make, store in a retrieved  
system and supply copies of this unpublished research if requested by  
other universities and research libraries.

By signing this form, I acknowledged that I have read and understand the IIUM  
Intellectual Property Right and Commercialization policy.

Affirmed by Alhassan Muhammad Alhassan

.....  
Signature

.....  
Date

*This thesis is dedicated to my father, Late Qadi Muhammad Alhassan who encouraged me to undertake this PhD and provided ample resources towards its accomplishment, but, as Allah so wished he did not survive to witness its completion. May Allah (SWT) have mercy on him and grant him Al-jannatul firdaus.*

## ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious, the Most Merciful. All praises and adoration are due to Allah alone (SWT) by whose mercy this work was accomplished. May His peace and blessings be upon Prophet Muhammad (SAW), his household, companions and all those that follow them with sincerity and piety.

I wish to express my sincere gratitude to my supervisor Assoc. Prof. Dr. Qamar Uddin Ahmed for his guidance, continuous support, encouragement and leadership, and for that, I will be forever grateful.

I am grateful to members of my supervisory committee and all those who provided their time, effort and support for this project.

I wish to express my profound appreciation to my dear mother; Hajiya Zainab, step mother; Hajiya Hadiza, wife; Hauwa, children; Khadija and Fatima, brothers; Aliyu, Aminu, Lukman and sisters; Hajiya Hadiza and Hajiya Rakiya. Your love, patience and support have been very wonderful and were vital to the successful achievement of this goal.

I also wish to express my gratitude to Usmanu Danfodiyo University Sokoto, Nigeria, for giving me the fellowship opportunity to undertake this study under the Tertiary Education Fund (Tetfund), 2013/2014 Intervention.

Finally, I am extremely indebted to the Ministry of Higher Education (MOHE), Malaysia and the Research Management Center, IIUM for providing financial support through Fundamental Grant Research Scheme (FRGS 13-089-0330), and Research Initiative Grant Schemes (RIGS 16-294-0458), respectively to accomplish this work.



# TABLE OF CONTENTS

Abstract .....	ii
Abstract in Arabic .....	iii
Approval Page.....	iv
Declaration .....	v
Copyright Page.....	vi
Acknowledgements .....	viii
Table of contents .....	ix
List of Abbreviations .....	xiii
List of Tables .....	xv
List of Figures .....	xvii
<b>CHAPTER ONE INTRODUCTION .....</b>	<b>1</b>
1.1 Background and Justification .....	1
1.2 Research Objectives.....	5
1.3 Research Hypotheses .....	5
1.4 Significance of the Study.....	6
<b>CHAPTER TWO LITERATURE REVIEW .....</b>	<b>7</b>
2.1 Medicinal Plants: An Overview.....	7
2.1.1 Drug Discovery from Medicinal Plants .....	7
2.1.2 Challenges of Drug Discovery from Medicinal Plants .....	13
2.1.3 Techniques in Drug Discovery from Plant .....	14
2.1.3.1 Chromatography .....	15
2.1.3.2 Nuclear Magnetic Resonance Spectroscopy.....	17
2.1.3.3 Mass spectrometry .....	18
2.1.3.4 Computational methods in drug discovery.....	21
2.2 Synthesis and Role of Plant Secondary Metabolites .....	23
2.2.1 Terpenoids.....	25
2.2.1.1 Monoterpenes .....	25
2.2.1.2 Diterpenes .....	25
2.2.1.3 Triterpenes .....	26
2.2.1.4 Sesquiterpenes .....	26
2.2.2 Phenolic Compounds .....	29
2.2.2.1 Flavonoids .....	30
2.2.3 Nitrogen Containing Compounds .....	33
2.2.3.1 Alkaloids.....	33
2.2.3.2 Cyanogenic glycosides .....	33
2.3 Pharmacological activity .....	35
2.3.1 Diabetes Mellitus .....	35
2.3.1.1 Diagnosis of diabetes mellitus.....	35
2.3.1.2 Prevalence of diabetes mellitus .....	37
2.3.1.3 Pathophysiology of type 2 diabetes .....	39
2.3.1.4 Role of obesity in insulin resistance .....	40
2.3.1.5 Genetic predisposition .....	41
2.3.1.6 Defects in insulin secretion and insulin action .....	41

2.3.1.7 Oral hypoglycemic agents .....	42
2.3.2 Cancer: The Role of Plant Based Natural Products .....	44
2.4 Dilleniaceae Family .....	45
2.4.1 Genus <i>Tetracera</i> .....	46
2.4.2 <i>Tetracera indica</i> (Christm. & Panz.) Merr. ....	46
2.5 Asteraceae Family .....	48
2.5.1 Genus <i>Gymnanthemum</i> .....	49
2.5.2 <i>Gymnanthemum glaberrimum</i> (Welw. ex O.Hoffm) H. Rob .....	50
2.6 Oxalidaceae Family .....	51
2.6.1 Genus <i>Averrhoa</i> .....	52
2.6.2 <i>Averrhoa bilimbi</i> Linn.....	52

**CHAPTER THREE PHYTOCHEMICAL INVESTIGATION AND ALPHA-GLUCOSIDASE INHIBITORY ACTIVITY OF *Tetracera indica* Christm. & Panz.) Merr. LEAVES .....**

<b>Christm. &amp; Panz.) Merr. LEAVES .....</b>	<b>55</b>
3.1 Introduction.....	55
3.2 Materials and Methods .....	56
3.2.1 Chemicals.....	56
3.2.2 Characterization and Spectroscopic Analysis .....	56
3.2.3 Phytochemical Tests .....	57
3.2.4 Isolation and Purification of compounds from the Leaves of <i>T. indica</i> .....	58
3.2.4.1 Plant collection and identification .....	58
3.2.4.2 Preparation of ethanol extract of <i>T. indica</i> leaves .....	58
3.2.4.3 Chromatographic fractionation of crude ethanolic extract .....	59
3.2.4.4 Chloroform fraction.....	60
3.2.4.5 EtOAc fraction.....	61
3.2.4.6 Methanol fraction .....	62
3.2.5 Alpha-glucosidase Inhibition Assay .....	63
3.2.5.1 Statistical analysis.....	63
3.2.6 Molecular Docking Studies.....	64
3.2.6.1 Software and programs.....	64
3.2.6.2 Receptor and ligand preparation.....	64
3.2.6.3 Molecular docking experiment.....	65
3.3 Results and Discussion .....	65
3.3.1 Isolation and Characterization of Bioactive Compounds of <i>Tetracera indica</i> .....	65
3.3.1.1 MAQ-1 (5,7-dihydroxy-8-methoxyflavone) (wogonin)...	65
3.3.1.2 MAQ-2 (3,5,7,4'-tetrahydroxyflavonol) (kaempferol) .....	68
3.3.1.3 MAQ-3 (3,5,7,3',4'-pentahydroxyflavonol) (quercetin) ...	71
3.3.1.4 MAQ-4 (5-hydroxyl-7-methoxyflavone) .....	75
3.3.1.5 MAQ-5 (5,7,8-trihydroxyflavone) (norwogonin).....	77
3.3.1.6 MAQ-6 (lupeol).....	80
3.3.1.7 MAQ-7 Betulinic acid .....	83
3.3.1.8 MAQ-8 (5,7-dihydroxyflavone-O-8-sulphate).....	86
3.3.1.9 MAQ-9 (5-hydroxy-8-methoxyflavone-O-sulphate).....	90
3.3.2 Alpha-glucosidase Inhibitory Activity Evaluation .....	94
3.4 Molecular Docking .....	96
3.5 Conclusion .....	104

<b>CHAPTER FOUR PHYTOCHEMICAL AND ANTIPROLIFERATIVE INVESTIGATIONS OF <i>Gymnanthemum glaberrimum</i> (Welw. ex O. Hoffm.) H. Rob</b> .....	<b>110</b>
4.1 Introduction.....	110
4.2 Materials and Methods .....	111
4.2.1 Collection and Identification of <i>G. glaberrimum</i> Leaves .....	111
4.2.2 Preparation of Methanol Extract of <i>G. glaberrimum</i> Leaves.....	111
4.2.3 Isolation and Purification of Bioactive Constituents .....	112
4.2.3.1 Methanol fraction .....	112
4.2.3.2 Hydrolysis of VMQ-3.....	113
4.2.3.3 Chloroform fraction.....	113
4.2.3.4 Hexane fraction.....	113
4.2.4 Characterization and Spectroscopic Analysis .....	114
4.2.5 <i>In vitro</i> Cytotoxic Evaluation.....	115
4.2.5.1 Cell lines and reagents.....	115
4.2.5.2 Cell culture techniques .....	115
4.2.5.3 Thawing cells.....	116
4.2.5.4 Sub culturing cells .....	116
4.2.5.5 Measuring cell viability using Trypan blue exclusion assay method (TBEA) .....	116
4.2.5.6 Seeding of cells for treatment.....	117
4.2.5.7 Determination of 50 % inhibitory concentration (IC50) using MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) cell proliferation assay.....	118
4.2.6 Statistical Analysis.....	119
4.2.7 Molecular Docking .....	119
4.3 Results and Discussion .....	120
4.3.1 Isolation and Characterization .....	120
4.3.1.1 VMQ-1 (Nonacosanoic acid).....	121
4.3.1.2 VMQ-2 (lupeol).....	123
4.3.1.3 VMQ-3 (5-Methylcoumarin-4- $\beta$ -glucoside).....	126
4.3.1.4 VMQ-4 (4-hydroxy-5-methylcoumarin) .....	130
4.3.2 Cytotoxicity Studies.....	132
4.3.3 Molecular Docking .....	136
4.4 Conclusion .....	149

<b>CHAPTER FIVE ANTIOXIDANT EVALUATION OF <i>Averrhoa bilimbi</i> LEAVES AND IDENTIFICATION OF BIOACTIVE CONSTITUENTS</b> .....	<b>151</b>
<b>THROUGH LC-MS</b> .....	<b>151</b>
5.1 Introduction.....	151
5.2 Materials and Methods .....	152
5.2.1 Materials.....	152
5.2.2 Plant Collection and Processing.....	152
5.2.3 Extraction and Fractionation.....	153
5.2.4 Preliminary Phytochemical Screening .....	153
5.2.5 LC-MS QTOF Analysis of Bioactive Constituents of n-Butanol Fraction .....	155
5.2.6 DPPH Free Radical Scavenging Activity .....	155
5.2.7 Xanthine Oxidase Inhibitory Activity Assay .....	156

5.2.8 Molecular Docking .....	157
5.2.9 Statistical Analysis .....	157
5.3 Results and Discussions.....	158
5.3.1 Antioxidant Capacities.....	158
5.3.2 DPPH Radical Scavenging Activity .....	159
5.3.3 Xanthine Oxidase Inhibitory Activity.....	160
5.3.4 LC-MS QTOF Analysis .....	162
5.3.5 Identification of Compounds through MS/MS Fragmentation Analysis .....	164
5.3.6 Molecular Docking Studies.....	171
5.4 Conclusion .....	174
<b>CHAPTER SIX CONCLUSION AND RECOMMENDATION.....</b>	<b>176</b>
6.1 Conclusion .....	176
6.2 Recommendations.....	177
<b>REFERENCES.....</b>	<b>179</b>
<b>Appendix 1 Spectra of MAQ-1 .....</b>	<b>196</b>
<b>Appendix 2 Spectra of MAQ-2 .....</b>	<b>203</b>
<b>Appendix 3 Spectra of MAQ-3 .....</b>	<b>210</b>
<b>Appendix 4 Spectra of MAQ-4 .....</b>	<b>217</b>
<b>Appendix 5 Spectra of MAQ-5 .....</b>	<b>223</b>
<b>Appendix 6 Spectra of MAQ-6 .....</b>	<b>230</b>
<b>Appendix 7 Spectra of MAQ-7 .....</b>	<b>237</b>
<b>Appendix 8 Spectra of MAQ-8 .....</b>	<b>244</b>
<b>Appendix 9 Spectra of MAQ-9 .....</b>	<b>251</b>
<b>Appendix 10 Spectra of VMQ-1 .....</b>	<b>259</b>
<b>Appendix 11 Spectra of VMQ-2 .....</b>	<b>264</b>
<b>Appendix 12 Spectra of VMQ-3 .....</b>	<b>271</b>
<b>Appendix 13 Spectra of VMQ-4 .....</b>	<b>278</b>
<b>Appendix 14 PUBLICATIONS .....</b>	<b>284</b>

## LIST OF ABBREVIATIONS

Ac	Acetone
A375 cells	Human malignant melanoma cells
$^{13}\text{C}$ -NMR	$^{13}\text{C}$ Carbon nuclear magnetic resonance
C	Carbon
CC	Column chromatography
$\text{CDCl}_3$	Deuterated chloroform
COSY	Homonuclear Correlation Spectroscopy
$\text{CHCl}_3$	Chloroform
2D	Two dimensional
dd	Doublet of the doublet
dL	Deci litre
DCM	Dichloromethane
DMSO	Dimethyl sulphoxide
DPPH	2, 2-diphenyl-1-1-picrylhydrazyl radical
EtOH	Ethanol
EtOAc	Ethyl acetate
$\text{FeCl}_3$	Ferric chloride
Fig.	Figure
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
$^1\text{H}$ -NMR	Proton nuclear magnetic resonance
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
HSQC	Heteronuclear single quantum correlation
HT-29 cells	Human caucasian colon adenocarcinoma cells
HMBC	Heteronuclear multiple bond correlation
Hz	Hertz
$\text{H}_2\text{SO}_4$	Sulphuric acid
IDDM	Insulin dependent diabetes mellitus
$\text{I}_2$	Iodine
<i>J</i>	Coupling constant
Kg	Kilogram
<i>m/z</i>	Mass-to-charge ratio
MeOD	Deuterated methanol
MeOH	Methanol
MCF-7 cells	Human breast adenocarcinoma cells
MS	Mass Spectrometry
<i>m</i>	Multiplet
Mg	Milligram
NMR	Nuclear magnetic resonance spectroscopy
NOESY	Nuclear overhauser enhancement spectroscopy
NIDDM	Non-insulin dependent diabetes mellitus
OGTT	Oral glucose tolerance test
prep.	Preparative
ppm	Parts per million

s	Singlet
t	Triplet
T:E:F	Toluene: Ethyl formate: Formic acid
TLC	Thin layer chromatography
UV	Ultraviolet
2D	Two dimensional

## LIST OF TABLES

<u>Table No.</u>		<u>Page No.</u>
2.1	Prevalence of diabetes and estimated diabetic people's numbers by region among adults aged 20–79 years for the years 2015 and 2040	38
3.1	$^1\text{H}$ and $^{13}\text{C}$ -NMR spectral values for MAQ-1	68
3.2	$^1\text{H}$ and $^{13}\text{C}$ -NMR spectral values for MAQ-2	71
3.3	$^1\text{H}$ and $^{13}\text{C}$ -NMR spectral values for MAQ-3	74
3.4	$^1\text{H}$ and $^{13}\text{C}$ -NMR spectral values for MAQ-4	77
3.5	$^1\text{H}$ and $^{13}\text{C}$ -NMR spectral values for MAQ-5	79
3.6	$^1\text{H}$ and $^{13}\text{C}$ -NMR spectral values for MAQ-6	82
3.7	$^1\text{H}$ and $^{13}\text{C}$ -NMR spectral values for MAQ-7	85
3.8	$^1\text{H}$ and $^{13}\text{C}$ -NMR spectral values for MAQ-8	89
3.9	$^1\text{H}$ and $^{13}\text{C}$ -NMR spectral values for MAQ-9	93
3.10	$\text{IC}_{50}$ for extracts and fractions of <i>Tetracera indica</i>	94
3.11	Alpha-glucosidase inhibitory activity of isolated compounds of <i>Tetracera indica</i>	96
3.12	Docking scores of $\alpha$ -glucosidase inhibitors	97
3.13	Physical properties of the isolated compounds	105
4.1	$^1\text{H}$ and $^{13}\text{C}$ -NMR spectral values for VMQ-1	122
4.2	$^1\text{H}$ and $^{13}\text{C}$ -NMR spectral values for VMQ-2	125
4.3	$^1\text{H}$ and $^{13}\text{C}$ -NMR spectral values for VMQ-3	129
4.4	$^1\text{H}$ and $^{13}\text{C}$ -NMR spectral values for VMQ-4	131
4.5	The $\text{IC}_{50}$ values for the cytotoxic activity of crude methanolic extract of <i>Gymnanthemum glaberrimum</i>	132
4.6	$\text{IC}_{50}$ values for 72 hours treatment with <i>Gymnanthemum glaberrimum</i> compounds on different cell lines	134

4.7	Docking result for VMQ-2 on CDK2	137
4.8	Docking score of compounds on carbonic anhydrase XII	143
4.9	Docking score of compounds on carbonic anhydrase IX	143
4.10	Physical properties of the isolated compounds	150
5.1	Phytochemical screening for chemical class identification of <i>Averrhoa bilimbi</i>	159
5.2	Free radical scavenging activity of crude methanolic extract of <i>Averrhoa bilimbi</i> and fractions	160
5.3	IC <sub>50</sub> for XO inhibitory activity of crude methanolic extract and fractions	162
5.4	Results of LC-MS Q-TOF analysis of n-butanol fraction	163
5.5	Docking scores of cucumerin A, afzelechin 3-O-alpha-L-rhamnopyranoside and acarbose on $\alpha$ -glucosidase	171



## LIST OF FIGURES

<u>Figure No.</u>		<u>Page No.</u>
2.1	Schematic representations of a typical medicinal plant drug discovery process	8
2.2a	Structures of some clinically useful drugs developed through medicinal plant drug discovery process	11
2.2b	Structures of some clinically useful drugs developed through medicinal plant drug discovery process	12
2.3	Generic schemes for isolation of natural products	17
2.4	Basic components in a mass spectrometer	19
2.5	Mevalonic acid pathways showing the synthesis of steroidal terpene-lanosterol	27
2.6	Structures of monoterpenes	28
2.7	Structures of some diterpenoids based compounds	28
2.8	Chemical structures of triterpenoids	29
2.9	Structure of main classes of flavonoids	31
2.10	Biosynthetic pathway for flavonoids	32
2.11	Different types of cyanogenic glycosides	34
2.12	Prevalence of diabetes mellitus in adults aged 18 years and above between 2006 and 2015	39
2.13	Photograph image of <i>Tetracera indica</i> leaves	48
2.14	Photograph image of <i>Gymnanthemum glaberrimum</i>	51
3.1	Structure of MAQ-1	67
3.2	Structure of MAQ-2	70
3.3	Structure of MAQ-3	73
3.4	Structure of MAQ-4	76

3.5	Structure of MAQ-5	79
3.6	Structure of MAQ-6	81
3.7	Structure of MAQ-7	84
3.8a	HMBC of MAQ-8	88
3.8b	Structure of MAQ-8	89
3.9	Structure of MAQ-9	92
3.10	HMBC of MAQ-9	92
3.11	Alpha-glucosidase inhibitory activity of crude extracts and fractions of <i>Tetracera indica</i> leaves	100
3.12	Surface structure of yeast $\alpha$ -glucosidase (PDB ID: 3A4A) with maltose posed in the catalytic pocket.	100
3.13	Binding interactions of MAQ-1 with active site residues of yeast $\alpha$ -glucosidase	101
3.14	Binding interactions MAQ-5 with active site residues of yeast $\alpha$ -glucosidase	101
3.15	Binding interactions of MAQ-2 with active site residues of yeast $\alpha$ -glucosidase	102
3.16	Binding interactions of MAQ-3 with active site residues of yeast $\alpha$ -glucosidase	102
3.17	Binding interactions of MAQ-8 with active site residues of yeast $\alpha$ -glucosidase	103
3.18	Binding interactions of acarbose with active site residues of yeast $\alpha$ -glucosidase	103
3.19	Surface structure of yeast $\alpha$ -glucosidase (PDB ID: 3A4A) with acarbose posed in the catalytic pocket	104
3.20	Flow chart for the preparation of ethanol extract and fractions from <i>Tetracera indica</i> leaves	106
3.21	Flow for isolation of compounds from chloroform fraction of <i>Tetracera indica</i> leaves extract	107
3.22	Flow chart for isolation of compounds from ethyl acetate fraction of <i>Tetracera indica</i> leaves extract	108
3.23	Flow chart for isolation of compounds from	

	methanol fraction of <i>Tetracera indica</i> leaves extract	109
4.1	Structure of VMQ-1	122
4.2	Structure of VMQ-2	124
4.3	Structure of VMQ-3	128
4.4	Structure of VMQ-4	131
4.5	Effect of methanolic leaves extract of <i>Gymnanthemum glaberrimum</i> on cancer cells viability as determined by MTS assay	133
4.6	Effect of <i>Gymnanthemum glaberrimum</i> compounds on the viability of A375 cell lines	136
4.7	Effects of <i>Gymnanthemum glaberrimum</i> compounds on the viability of HT-29 cell lines	136
4.8	Active site residues of CDK2	138
4.9	Binding interactions of active site residues of CDK2 (PDB ID: 2DUV) with VMQ-2	140
4.10	Binding interactions of active site residues of CDK2 with 2-(3,4-dihydroxyphenyl)-8-(1,1-dioxidoisothiazolidin-2-yl)-3-hydroxy-6-methyl-4h-chromen-4-one (co-crystallized)	140
4.11	Surface structure of CDK2 showing the binding pose of VMQ-2	141
4.12	Active site residues of CAIX (PDB ID 3IAI)	142
4.13	Binding interactions of active site residues of CAIX with VMQ-3	144
4.14	Binding interactions of active site residues of CAIX with VMQ-4	144
4.15	Binding interactions of active site residues of CAIX with 5-acetamido-1,3,4-thiadiazole-2-sulfonamide (co-crystallized ligand)	145
4.16	Binding interactions of active site residues of CAXII (PDB ID 4HT2) with VMQ-3	146
4.17	Binding interactions of active site residues of CAXII with VMQ-4	147

4.18	Binding interactions of active site residues of CAXII (PDB ID 4HT2) with 4-[(4,6-dimethylpyrimidin-2-yl)thio]-2,3,5,6-tetrafluorobenzene sulphonamide (co-crystallized ligand)	147
4.19	Flow chart for isolation of bioactive compounds of <i>Gymnanthemum glaberrimum</i> leaves extract	150
5.1	Structure of 5,7,4'-trihydroxy-6-(1-ethyl-4-hydroxyphenyl)flavone-8-glucoside (Cucumerin A)	164
5.2	Structure of Afzelechin 3-O-alpha-L-rhamnopyranoside	164
5.3	MS/MS spectrum of 5,7,4'-trihydroxy-6-(1-ethyl-4-hydroxyphenyl)flavone-8-glucoside (Cucumerin A)	167
5.4	MS/MS spectrum of Afzelechin 3-O-alpha-L-rhamnopyranoside	168
5.5	Fragmentation pattern of 5,7,4'-trihydroxy-6-(1-ethyl-4-hydroxyphenyl)flavone-8-glucoside (cucumerin A)	169
5.6	Fragmentation pattern of Afzelechin 3-O-alpha-L-rhamnopyranoside	170
5.7	Binding interactions of 5,7,4'-trihydroxy-6-(1-ethyl-4-hydroxyphenyl)-flavone-8-glucoside (cucumerin A) with active site residues of yeast $\alpha$ -glucosidase (PDB ID 3A4A).	173
5.8	Binding interactions of afzelechin 3-O-alpha-L-rhamnopyranoside with active site residues of yeast $\alpha$ -glucosidase (PDB ID 3A4A)	174

# CHAPTER ONE

## INTRODUCTION

### 1.1 BACKGROUND AND JUSTIFICATION

Diabetes mellitus and cancer are two major diseases with huge impact on health worldwide. They contribute significant percentage to the overall number of deaths associated with non-communicable diseases (WHO, 2016).

Diabetes mellitus is a complex metabolic disorder characterized by increased blood glucose level resulting from insulin inadequacy or defect in insulin action or both. It affects both the young and the old, and it occurs in all regions of the world (Jaacks et al., 2016; Zimmet, 2017). Most cases of diabetes mellitus are grouped under two main categories namely; type-1 and type-2 diabetes. Type-1 diabetes is also known as insulin-dependent diabetes mellitus (IDDM), in which the pancreatic  $\beta$ -cells do not produce insulin at all, mostly befalls in children and young adults. It accounts for 5-10% of diabetes (Maahs et al., 2010). Type-2 diabetes is also known as non-insulin dependent diabetes mellitus, in which the pancreatic  $\beta$ -cells fail to produce sufficient amount of insulin or the body lacks the ability to properly utilize insulin. It is considered the most common form of the disease accounting for 90-95% of total cases of diabetes worldwide (Maahs et al., 2010). Diabetes mellitus has become a major public health problem across the world and is considered a key contributor to the global burden of diseases and disabilities. Its prevalence has been increasing rapidly due to obesity and sedentary life styles (Malik et al., 2013). It is considered as one of the largest epidemics in human history (Zimmet, 2017). According to the International Diabetic Federation (IDF, 2015), about 415 million

people are affected by the disease worldwide and the number is set to further increase beyond 600 million by 2040. Type-2 diabetes mellitus is one of the major diseases affecting Malaysians. Its incidence and prevalence is growing steadily. The prevalence of type-2 diabetes among adults in Malaysia (aged  $\geq 18$  years) has increased from 8.3% in 1996 to 11.6% in 2006 and subsequently to 15.2% in 2011 (Wan Nazaimoon et al., 2013). The Malaysian National Health Morbidity Survey conducted recently in 2015, recorded an overall diabetes prevalence of 17.5% (aged  $\geq 18$  years) (NHMS, 2015). Although tremendous progress has been made in the area of oral hypoglycemic agents, there are still unmet clinical needs with respect to the prevention of type-2 diabetes in high risk individuals, control of blood glucose levels and risk of developing long term complications (Bennett et al., 2014). The major drawbacks of the conventional agents used in the treatment of type-2 diabetes mellitus are frequent decrease in efficacy overtime leading to inadequate glycemic control as well as drug related adverse effects (Bennett et al., 2014). Hence, there is an urgent need for more researches to discover newer and more effective drugs to overcome diabetes.

Cancer is another important disease that constitutes a major public health problem. Cancer is a life threatening and debilitating incurable malady characterized by the abnormal rapid proliferation of cells that invade and destroy other tissues. It accounts for high number of morbidity and mortality associated with non-communicable diseases worldwide. It is the second leading cause of death after cardiovascular disorders. The total number of people living with cancer is growing steadily due to increase in population as well as exposure to risk factors such as smoking, poor diet, physical inactivity, reproductive changes and environmental pollution (Torre et al., 2015). Recent report has shown that, there were

approximately 14 million new cases and 8.2 million cancer related deaths in 2012, and more than 60% of the world's total new annual cases occur in Africa, Asia and Central and South America (Torre et al., 2015). These regions account of 70% of the world's cancer deaths. There are different types of cancers depending on the tissue or organ affected. The most common among them include cancer of the brain, lungs, breast, colorectal, skin, cervix, bladder and prostate. Today, cancer is one of the leading causes of mortality in Malaysia. Recent report from Malaysian cancer registry shows that, a total number of 103,507 new cancer cases were diagnosed during 2007 to 2011. About 45.2% of cases were reported in males while females have 54.8%. The five most common cancers among Malaysian males were cancers of the colorectum, lung, nasopharynx, lymphoma and prostate while cancers of the breast, colorectal, cervix uterus, ovary and lung are the most common among females (Azizah et al., 2016).

Plants used in folklore medicine represent an important source of biologically active compounds that could help to meet our current need for better anti-diabetic and anticancer drugs. According to the World Health Organization (WHO), large percentage of people living in Africa and Asia use the traditional or complementary alternative medicine to help meet some of their main healthcare needs. People in Europe, Australia, and United States have been increasingly embracing the use of traditional herbal medications to complement orthodox medicine (Qu et al., 2014; Rounds et al., 2017). Cumulative evidence from research with medicinal plants has revealed that their bioactivity is not due to a single chemical entity but often results from synergistic effect of multiple constituents, which implies that the secondary metabolites in a plant extract that are derived from their diverse chemistry, can act on

different and multiple targets involved in the pathogenic process, to augment overall therapeutic efficacy (Yang et al., 2014).

In spite of the great development in synthetic and combinatorial chemistry techniques, natural products remain an important source of molecules for discovery of new drug entities. For example, detailed investigation of new drugs approved by the US Food and Drug Administration (FDA) between 1981 and 2010 has shown that 34% of those drugs that were based on small molecules were either natural products or their direct derivatives including; hypolipidemics, anticancer drugs and immune suppressants (Newman and Cragg, 2012). From around the 1940s to the end of 2014, of all the 175 small molecules approved for cancer therapy, 85 (49%) are either natural products or their semi-synthetic derivatives (Newman and Cragg, 2016). The above analysis emphasizes the importance of natural product as source of valuable drugs for treatment of diseases and enhancement of general well-being. The discovery of drug from a medicinal plant often starts from the knowledge of its application in folk medicine, which is then subjected to pharmacological investigation to ascertain the authenticity of the claim. The natural product is required as a pure and characterized compound for further pharmacological investigation. This is achieved through bioactivity guided isolation and characterization using spectroscopic techniques.

In view of the foregoing, this research was focussed on the phytochemical and pharmacological investigations of *Tetracera indica*, *Averrhoa bilimbi* and *Gymnanthemum glaberrimum* leaves extracts which are claimed to be useful in the traditional treatment of diabetes mellitus and cancer but their phytoconstituents have not been well studied.